

Appl. No. : 10/743,991 Confirmation No. 9031  
Applicant : D. Michael Connolly  
Filing Date : December 23, 2003 Art Unit: 1637  
Title : IDENTIFYING ITEMS WITH NUCLEIC ACID TAGGANTS  
Examiner : WOOLWINE, Samuel C.  
Docket No. : 3027914  
Customer No. : 44,331

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA. 22313-1450

Sir:

**DECLARATION PURSUANT TO 37 C.F.R. 1.130**

I, D. Michael Connolly, state that I am the sole inventor in the above-referenced patent application. Said application is owned by Integrated Nano-Technologies, LLC by virtue of an Assignment which was recorded with the United States Patent and Trademark Office on May 10, 2004 under Reel/Frame 015307/0212 in the name of Integrated Nano-Technologies, LLC.

Additionally, U.S. Patent No. 6,399,303; U.S. Patent No. 6,593,090; U.S. Patent Application Publication No. 2003-0203384; and U.S. Patent Application Publication No. 2003-0109031 are all owned by Integrated Nano-Technologies, LLC.

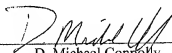
I am a named inventor in each of these patents and/or patent applications.

I declare that I am the prior inventor under 35 U.S.C. 104.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further

that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 6/29/09

  
D. Michael Conrilly

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Sir:

**DECLARATION PURSUANT TO 37 C.F.R. 1.131**

I, D. Michael Connolly, state that I am the sole inventor of the claimed subject matter of the above-referenced patent application, hereinafter referred to as the invention.

I have read and am familiar with Childers et al. U.S. Patent Application Publication No. 2004/0086872, published May 6, 2004, cited by the Examiner.


Prior to October 31, 2002, I conceived of and actually reduced to practice the claimed invention. This is demonstrated by the submission of the write up containing working examples demonstrating the detection of DNA taggants.

Exhibit A is a copy of an electronic disclosure written prior to October 31, 2002 describing the making and detection of DNA taggants, which is disclosed in the above-referenced patent application.

I hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title

18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 6/17/2009

  
D. Michael Connolly

## **Exhibit A**

## **ABSTRACT**

## **BACKGROUND**

Forgery of branded materials has a large impact for companies who lose revenue due to competition from counterfeit products; for customers who may have safety concerns associated with poor quality counterfeit goods; and for governments which may lose tax revenues. Anti-counterfeiting technologies are currently used in every conceivable product including licensed clothing, computers, software, electrical goods and consumable products. Additionally, the tracking of explosives and materials used to produce explosive devices is a major concern for law enforcement agencies worldwide.

Current anti-counterfeiting technologies include security printing with special watermarks, inks and dyes, holograms, tamper proof labels, magnetic and RFID tags. While all these methods are effective to some extent, none is completely counterfeit-proof. In contrast, the incorporation of cloaked DNA (or other oligonucleotide) taggants into a product or its packaging provides a virtually counterfeit-proof method of determining the authenticity and source of the material.

Analysis of nucleic acids, such as deoxyribonucleic acids (DNA) or ribonucleic acids (RNA), for clinical and forensic uses has become a routine procedure. For instance, molecular biology techniques allow detection of congenital or infectious diseases based on nucleic acid sequences. These same techniques can also characterize DNA for use in settling factual issues in legal proceedings such as paternity suits and criminal prosecutions. DNA testing has been made possible due to amplification methods. One can take small amounts (theoretically a single molecule) of DNA which, in and of itself would be undetectable, and increase or amplify the quantity present to a degree where an amount sufficient for detection is present. This amplification has been made possible by the widely used technique known as polymerase chain reaction (PCR).

US Patent No. 5,139,812 describes a method of secretly marking moveable property with a small amount of a known DNA molecule. The DNA can be that of the owner of the property. Provided the mark is made in secret, and is not visible, proof of ownership can be established by amplifying the DNA, reading the DNA sequence of base pairs, and showing that it corresponds with that of the owner. However, it would be relatively simple for a counterfeiter to apply a mark of another DNA to the property.

US Patent No. 6,312,911 describes a method of DNA-based steganography, wherein a DNA sequence corresponding to a coded message is inserted into genomic DNA. Because of the complexity and size of genomic DNA, there is no good way to determine which portion of the genome is the message, unless one possesses the key, i.e., the knowledge of where to look for the message.

Although it is relatively simple to tag a product or its packaging with a mark or label consisting of an oligonucleotide such as DNA, the marking is only useful as a tracking/anti-counterfeiting tool if it can be read easily and quickly by authorized personnel in a secure manner. The major flaw with current DNA-taggant systems is that the equipment needed to read the code is not portable, requires skilled operators and takes hours/days to provide a response. This document describes an oligonucleotide-taggant system that allows rapid, on-site decoding to be performed by a non-technical operator.

## **SUMMARY**

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## **DETAILED DESCRIPTION**

### **Taggant identity**

The described technology relies both on the vast number of possible code combinations that can be achieved with a limited number of oligonucleotide tags and on cloaking with irrelevant oligonucleotide fragments to preserve the security of the code. Selecting one or more members of a set of  $N$  objects can be done in  $2^N$  minus one different ways. Thus, a set of 32 different target molecules can yield up to 4,294,967,295 alternative combinations. If the molecules making up the code are mixed with a random selection of similar oligonucleotides it will be impossible for a counterfeiter to determine which molecules make up the code.

In one embodiment of the invention the 32 different target molecules are short segments (approximately 10 to 30 nucleotide bases) of an oligonucleotide such as deoxyribonucleic acid (DNA), but also including: ribonucleic acid (RNA); peptide nucleic acid (PNA); locked nucleic acid (LNA) or any other synthetic oligonucleotide.

In operation of this invention, a subset of the 32 target DNA molecules are selected and mixed with another number of portions of unknown, or random, DNA molecules. The mixture is then applied to an item of movable property, by methods discussed below. When authentic identification of the item of movable property is desired, the applied mixture of DNA is sampled and analyzed by known methods, discussed below. The detector reports the presence of any of the 32 known DNA molecules, but does not recognize any others. Thus, if the originally selected subset of DNA molecules is present, authenticity is confirmed.

Selection of the code (i.e., subset of DNA molecules) by the user, combined with a detector which recognizes the entire set of molecules, is an added security feature. The code would be set by the company manufacturing the product rather than the company

supplying the complete set of oligonucleotides. Security of the code would therefore be the responsibility of the users and the code could be altered at their convenience without prior notice to a second party.

Oligonucleotides can be chemically synthesized or produced by standard molecular biology techniques. For instance, a DNA target sequence could be inserted into a bacterial plasmid. This plasmid would then be cloned into *E. coli* and a large quantity of these bacteria grown in liquid culture. The plasmid would be isolated from the bacterial culture and digested with restriction enzymes to yield a mixture of DNA containing both the target sequence and a large quantity of random, cloaking DNA.

## **Marking**

In order to make a useful mark, the DNA should be applied in a way that is stable to ambient storage or shipping conditions. For example, if the item being marked is to be left out in the weather, the DNA should be applied in a waterproof matrix. Similar considerations would apply to temperature, pH, corrosive gases, and electromagnetic radiation. On the other hand, the DNA should be applied in such a way that it is easy to remove a sample for analysis. For example, this could be done by making the matrix soluble in a solvent contained on a swab. A number of marking methods are described below:

### Printing

The DNA labeling mixture can be directly printed onto packaging boxes or the articles themselves using a wide variety of techniques (Figure A). DNA can be incorporated into a toner formulation and printed onto the work by electrophotography, or it can be formulated into a water based ink, and printed by an inkjet printer. Flexo printing uses a rubber impression cylinder with raised areas where printing is desired. The raised areas are inked, usually with a water based ink, and the ink is transferred to the work piece by light pressure contact.

Printing can also be done with a gravure press, wherein the printing cylinder has indentations corresponding to the printed area. The indentations are filled with ink, and the excess ink is removed with a skiving blade. The printing cylinder is then contacted to the work, and the ink is transferred by capillary action from the cylinder to the work. In the case of DNA formulated into an oil soluble ink, an offset lithographic press can be used. In this case the printing plate has oleophilic areas where ink is desired, and a hydrophilic background. A water based fountain solution coats the background areas with water, which repels the ink, and the ink coats the oleophilic areas. Then the ink image is transferred to an intermediate cylinder, commonly called the blanket cylinder, and from the blanket cylinder to the work.

Other printing methods are: silk screen, where the ink is forced through an image on a fabric carrier by a squeegee and the background portions of the image fabric are blocked by a polymeric photoresist; tampon printing, where the image ink is carried on a



soft rubber tamp which is pressed onto the workpiece; or pin spot printing, where the image ink is picked up by capillary force into a hollow pin, and then contacted to the work piece where a portion of the ink is deposited.

#### Marking by hand

DNA ink similar to those used for printing can also be used in a fountain pen, a felt tipped pen, or a ball point pen to mark an item of portable property by hand (Figure B). Such marks could be visible (a pigment is added to the DNA mixture) or invisible and could be used to rapidly mark items at point of production. For example, an artist could use such an ink to "sign" a work with a unique signature that could not be forged (the painter, Thomas Kinkade already uses this technology to mark his artwork using paint containing his own DNA) or a baseball player could autograph a ball using a DNA-containing ink. Such a rapid marking system could be used to add a further level of security to packaged items, for example a covert mark could be superimposed on a pre-printed label or barcode as an item exits the production facility. The addition of such a mark would require no specialized equipment or expertise and the code could be frequently changed for additional security simply by supplying a new batch of DNA ink.

#### Incorporation of taggants into packaging materials

In the case of porous materials, e.g., wood, cardboard or paper, the oligonucleotide solution could be incorporated into the packaging by: injection with a syringe; infusion; or pressure treatment. Additionally, for both porous and non-porous materials (e.g., plastic film), the oligonucleotides could be incorporated into the packaging material during manufacture (Figure C). Samples for taggant analysis would be taken either by destroying a small portion of the packaging material, or by using a swab to lift some taggant from the surface. Such a use would make it impossible to remove the tag from the packaging material and would mean that any part of the package could be checked for the presence of the taggant.

#### Incorporation of taggants into labels

In one embodiment of the invention, a clothing label is imbibed with an oligonucleotide solution and allowed to dry (Figure D). This was used at the Sydney Olympics to prevent import/sale of counterfeit souvenir merchandise. Such a tag is unlikely to survive normal laundering of the clothing, but this is not a problem if the purpose of the taggant is to prevent import/sale of the items rather than prove its identity in the future.

In another embodiment of the invention, the DNA is dissolved in a solvent such as dimethylformamide along with a water insoluble polymer such as polyvinylbutryal. When dried, such a mixture will not be soluble in water, and thus can be washed without losing the taggant. For analysis, the DNA extracted by the same solvent can be precipitated by the addition of ethanol, spun down with a centrifuge and redissolved in buffer for electrophoresis.

Taggants could also be incorporated into paper/plastic labels during their manufacture so that sampling from any part of the label would reveal the taggant or added to the surface after production so that they could be sampled by a swab test (Figure E).

#### *Incorporation of taggants into label adhesives*

Another alternative for marking is to add oligonucleotides to the adhesive used to attach labels to packaging. Traces of such a mark could remain even if the label was removed and would be ideal for incorporation into a tamper-evident label or as an additional, covert, level of security.

#### *Incorporation of taggants into the product*

Marker oligonucleotides could be added to the product itself. This would allow any analysis of a sample of the product to show both its identity and source. Such an application would be useful in the case of explosives or of very valuable liquid products such as drugs, perfume or liquor. In the case of substances which are consumed, a DNA marker has the added advantage of being non-toxic. A taggant added to products such as oil or chemicals would also aid in tracing spills as the identity of the taggant would definitively indicate the owner/shipper of the substance spilled and could allow the government to assign responsibility for cleanup costs and/or any appropriate fines. The EPA is currently using microtaggant identification particles consisting of a color code to document the illegal transport and disposal of hazardous and regulated waste (U.S. EPA, 2002), but the oligonucleotide marking system described herein would offer the advantage of rapid onsite identification which is impracticable with the microtaggant system currently in use.

### **Tamper evident marking**

This technology could also be used to make a mark which will show that a package has been opened. In this case, an unstable oligonucleotide e.g., RNA is placed within a protective container on the packaging. If the packaging is opened the integrity of the protective container is breached and the RNA degraded so that it is no longer recognized by the detection system (Figure F). Such a mark would prevent reuse of legitimate packaging as a cover for counterfeit products and could provide evidence that a product has been tampered with. For example, boxes which contained designer jewelry are retrieved for reuse with cheaper counterfeit merchandise. However, the boxes contained an RNA tag which was destroyed when the original articles was removed and thus, they will no longer be recognized as containing genuine merchandise.

### **Choice of matrix**

The matrix for the DNA may be a polymeric material. In a preferred embodiment of the invention, the polymer is polyvinylalcohol, from 88 to 100% hydrolyzed. This polymer is, by virtue of crystalline structure, insoluble in cold water and all organic solvents, but soluble in hot water. Thus it provides a stable environment for storage of

the applied DNA, but can be removed for sampling with hot water on a swab. The polyvinylalcohol can be cross-linked with boric acid to provide even more sample integrity.

Many other polymers can be used as a matrix for DNA. For example, polyethyleneglycol, polyethylencimine, polyvinylpyridine, hydroxyethylcellulose, polyvinylbutyral, polyvinylpyrrolidone, polyvinylimidazole, and co-polymers of any of the aforementioned polymers could be used as matrixes.

## Detection

The use of DNA as a property marker has been previously proposed (for example, US Patent No. 5,139,812). However, the routine use of oligonucleotides such as DNA for tagging of merchandise is dependent on the ability to examine the taggant quickly and easily without having to send samples to a laboratory for analysis. Current state-of-the-art DNA detection technologies rely on the use of fluorescent dyes or radioactive tags to identify hybrid formation. However, thousands of binding events are needed before these signals are detectable, and thus a DNA amplification process is generally required. Amplification processes require a skilled technician, and are cumbersome, error prone and slow. In addition, such systems are not readily portable and cannot simultaneously detect multiple agents.

As part of the described tagging system, we propose the use of BioDetect™ (US Patent Application Serial # 09/545,010, Title, submitted on XXth XX, 2001, which is hereby incorporated by reference), a portable device which will allow rapid detection of target oligonucleotides contained within complex mixtures and can be operated by a non-specialist user. This device is based on technology described in U.S. Patent No. 6,248,529 “Chemically Assembled Nano-Seale Devices” which is hereby incorporated by reference.

BioDetect™ uses oligonucleotide hybridization to identify target sequences. Samples are processed within the device to produce a solution of oligonucleotide fragments which is analyzed for the presence of a specific sequence. Two probes (small pieces of oligonucleotide of known sequence) are attached to the surface of a microchip so that they are close together but not in contact. The probes are designed in two sets so that each set contains sequences complementary to opposite ends of a target oligonucleotide. Target oligonucleotide is extracted from a sample and deposited onto the microchip where it binds to the two sets of probes forming a link between them. Subsequent chemical treatment of the chip leads to the formation of a circuit wherever the target oligonucleotide is bound. Passage of electrical current through this circuit signals the presence of a specific target oligonucleotide molecule. The most significant feature of this device is its ability to accurately and rapidly detect the presence of a particular oligonucleotide sequence within a complex mixture even though the target sequence may be very rare.

The BioDetect™ device will consist of an electronic detector and single-use test cartridges. Single-use cartridges prevent sample cross contamination, provide a sealed archival chamber for further sample analysis, and will be stable at room temperature. Operation will not require specialized molecular biology knowledge and the system will tolerate samples containing suspended organic and inorganic particulates and a wide range of organic and inorganic solutes. Samples will require minimal pre-processing by filtration to remove particulate debris. The device will identify the presence of known oligonucleotide sequences from samples including: swabs of ink from printed labels; packaging material, the adhesive from a label; or the product itself. Cartridges will be made of plastics that accommodate both organic and inorganic solutes and can be customized to suit a particular user's needs.

The advantages of this system over those currently available are that it is rapid and can be performed onsite by a non-technical operator. Samples do not need to be sent to a laboratory for analysis by trained personnel. A consignment of merchandise can be checked at any point during shipping and a definitive determination of its authenticity made 15-30 minutes after sampling.

While the preceding description of the invention describes the practice of the invention, it should be made clear that any extension of these ideas and procedures is also part of this invention.

### **Examples**

A solution of 12 microliters of ladder DNA ( ) was mixed with 50 microliters of 2.5% polyvinylalcohol (98% hydrolyzed). Portions of 15 microliters of the solution were spotted onto a glass microscope slide, a cardboard box, and a piece of filter paper and allowed to dry. To simulate rain, the glass microscope slide was run under cold tap water for 10 seconds. After 24 hours, the samples on the microscope slide and the cardboard were recovered by dissolving in 15 microliters of warm water. The sample on filter paper was used as is. All three samples were placed in the wells of an agarose electrophoresis gel in TBE buffer. A 70-volt bias was applied to the gel for 2 hours. The gel was stained with Sybr-Gold dye and photographed under UV light. Fluorescent bands of the DNA ladder were clearly visible in all three samples and in the control run in the fourth lane. This example shows the utility of marking portable property with samples of DNA and recovering them at a later time to prove the identity of the property.

A Tampo printing stamp was prepared by placing a small dab of silicone calking compound (General Electric clear silicone calk) on a wooded paint stirrer curing overnight. The top of the calk dab was sliced off smoothly with a razor blade to create a printing surface. The flat surface of the calk dab was dipped into a solution of ladder DNA (100 base pair DNA ladder from Promega, Madison, Wisconsin), and then pressed against a glass microscope slide. The transferred DNA "ink" was allowed to dry. The dry DNA was wiped from the glass slide with a wet cotton swab and the sample removed from the swab by centrifugation at 10,000 rpm for 30 seconds. The DNA ladder was

identified by agarose electrophoresis, staining the gel with Sybr-Gold dye and observing the DNA bands by fluorescence.

A capsule of Prozac (Eli Lilly, Indianapolis, IN) was opened and one microliter of ladder DNA (100 base pair DNA ladder from Promega, Madison, Wisconsin) was spotted onto the contents of the capsule and allowed to absorb into the drug (Figure G). The capsule was closed. Although the tagged capsule was visually indistinguishable from un-tagged capsules, the contents could be identified by DNA detection.

## **CLAIMS**

SEP

## **REFERENCES**

U.S. EPA. 2002. The U.S. EPA National Enforcement Investigations Center. Accessed April 2002 online at: <http://es.epa.gov/oeca/ocft/neic/hilite1.html>.

US Patent No. 5,139,812

U.S. Patent No. 6,248,529 "Chemically Assembled Nano-Scale Devices"

US Patent No. 6,312,911

US Patent Application Serial # 09/545,010, Title - BioDetect™, submitted on XXth XX, 2001

## FIGURE LEGENDS

- A) DNA tag printed onto surface of product/packaging.
- B) Marking by hand with a pen containing DNA ink.
- C) DNA taggant incorporated into packaging material.
- D) Clothing label impregnated with DNA taggant.
- E) Bar code label to which DNA taggant has been applied.
- F) Tamper-evident label containing an RNA taggant which degrades when the package is opened.
- G) Addition of DNA taggant to the contents of a drug capsule.